The *AIB1* Polyglutamine Repeat Does Not Modify Breast Cancer Risk in *BRCA1* and *BRCA2* Mutation Carriers

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Abstract

This is by far the largest study of its kind to date, and further suggests that *AIB1* does not play a substantial role in modifying the phenotype of *BRCA1* and *BRCA2* carriers. The *AIB1* gene encodes the AIB1/SRC-3 steroid hormone receptor coactivator, and amplification of the gene and/or protein occurs in breast and ovarian tumors. A CAG/CAA repeat length polymorphism encodes a stretch of 17 to 29 glutamines in the HR-interacting carboxyl-terminal region of the protein which is somatically unstable in tumor tissues and cell lines. There is conflicting evidence regarding the role of this polymorphism as a modifier of breast cancer risk in BRCA1 and BRCA2 carriers. To further evaluate the evidence for an association between *AIB1* glutamine repeat length and breast cancer risk in *BRCA1* and *BRCA2* mutation carriers, we have genotyped this polymorphism in 1,090 *BRCA1* and 661 *BRCA2* mutation carriers from Australia, Europe, and North America. There was no evidence for an increased risk associated with AIB1 glutamine repeat length. Given the large sample size, with more than adequate power to detect previously reported effects, we conclude that the *AIB1* glutamine repeat does not substantially modify risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers. (Cancer Epidemiol Biomarkers Prev 2006;15(1):76–9)

Introduction

The *AIB1* (*NCOA3*) gene encodes the AIB1/SRC-3 steroid hormone receptor coactivator, and amplification of the gene and/or protein occurs in breast and ovarian tumors (1-4), and is associated with tumor size (2), immunohistochemical profile (including estrogen receptor, progesterone receptor, p53, and HER2 status; ref. 5), and tamoxifen resistance (6). A CAG/CAA repeat length polymorphism encodes a stretch of 17 to 29 glutamines in the HR-interacting carboxyl-terminal region of the protein, and although repeat number has not been directly assessed with respect to its effects on function, the repeat

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region has been shown to be somatically unstable in tumor tissues and cell lines. One study found that germ line DNA from *BRCA1/2* carrier cases have a greater proportion of uncommon sequence patterns compared with normal controls, and a greater proportion of alleles ≥28 repeats compared with sporadic breast cancer cases (7).

Several studies have been undertaken to assess the role of the *AIB1* glutamine repeat polymorphism as a modifier of breast cancer risk in *BRCA1* and *BRCA2* carriers, with the hypothesis-generating study of 448 female *BRCA1* or *BRCA2* mutation carriers reporting increased breast cancer risk associated with allele length ≥29 glutamines [odds ratio, 2.9; 95% confidence interval (CI), 1.7-5.0], an effect which appeared to be driven by the 370 *BRCA1* mutation carriers in the sample (8). Longer repeat length was associated with modestly increased risk in a second study of 222 *BRCA1* and 88 *BRCA2* mutation carriers [rate ratio (RR) per repeat 1.25 (95% CI, 1.1-1.4) for *BRCA1* carriers, and 0.9 (0.8-1.1) for *BRCA2* carriers; ref. 9], but not in another much larger study of 851 *BRCA1* and 324 *BRCA2* mutation carriers [RR per repeat 1.1 (95% CI, 0.8-1.3) and 1.2 (0.9-1.6) for *BRCA1* and *BRCA2* carriers, respectively; ref. 10].

To further evaluate the evidence for an association between *AIB1* glutamine repeat length and breast cancer risk in *BRCA1* and *BRCA2* mutation carriers, we have genotyped this polymorphism in a series of 1,754 *BRCA1* and *BRCA2* mutation carriers.

Materials and Methods

Subjects. The distribution of samples according to source, gene, and cancer status is shown in Table 1. Recruitment and genetic studies were approved by relevant ethics committees at all sites, and written informed consent was obtained from each participant. Mutation carriers were identified as part of clinic-, community-, multiple-case family-, and population-based research studies, as described elsewhere (11-15). Mutation classification was as described previously (11). A small subset of 17 individuals from the Australian Breast Cancer Family Study were also analyzed as part of a previous population-based case control study of *AIB1* (16).

Molecular Methods. The *AIB1* glutamine repeat length was measured by standard fluorescent PCR PAGE methodology, using the ABI Prism 373 Genescan and Genotyper systems. PCR primers used were F primer 5′-CCGACAACA-GAGGGTGGCTAT-3′, and R primer 5′-CTGGGGGAAG-CAGTCACATTAG-3′. The annealing temperature was 63°C. The *AIB1* glutamine repeat length was assayed in the Quebec samples by standard ^{35}S-dATP PCR. PCR primers used were F primer 5′-TCCGACAACAGAGGGTGGCTATG-3′, and R primer 5′-TTAGGAGGTGGGCTGAAGGCCTG-3′. The annealing temperature was 60°C.

Statistical Methods. Subject status characterization, potential confounder categorization, and statistical analysis methods have been described previously (11), with subjects grouped by country or origin (Table 1). Briefly, the primary analyses of association between genotype and disease risk were done using Cox regression with time to breast cancer onset as the end point. Repeat length was defined as either: (*a*) a binary variable, defined by stated cutpoints, (*b*) a continuous variable, using the length of the smaller of the two alleles, the larger of the two alleles, or the average length of a subject's two alleles. Confidence limits for the RR were calculated using a robust variance approach to allow for the dependence among individuals in the same family (17). Secondary analyses used the weighted Cox regression approach (11, 18), in which individuals were weighted such that observed breast cancer incidences in the study sample are consistent with established breast cancer risk estimates for *BRCA1* and *BRCA2* mutation carriers (19). R version 1.9.0 was used for all analyses. S-Plus VI was used for power calculations, as described previously (11, 18).

Results

Genotype distributions were similar to those in previous studies. The glutamine length ranged from 18 to 37 repeats, the most common alleles being 26 repeats (13%), 28 repeats (38%), and 29 repeats (47%). The estimated RRs associated by repeat length are given in Table 2. There was no evidence for an increased risk associated with *AIB1* glutamine repeat length, for the ≥28 and ≥29 repeat cutpoints previously shown to be associated with risk (8), or for repeat length considered as a continuous variable. None of the estimated RRs were different from 1 at the 0.05 level of significance, for *BRCA1* or *BRCA2* mutation carriers. There was little difference between the estimates adjusted only for source group, ethnicity, and year of birth, and those

Table 1. Characteristics of study subjects

Sample sources*	Mode of ascertainment	Grouping	*BRCA1*	*BRCA2*	BRCA1 and *BRCA2*
			n (% of total)	*n* (% of total)	*n*
EMBRACE	clinic-based	United Kingdom	386 (35.4)	175 (26.5)	
kConFaB	clinic-based	Australia	237 (21.7)	217 (32.8)	
BCFR-Australia-AJBCS	community-based	Australia	18 (1.7)	22 (3.3)	
BCFR-Australia-ABCFS	population-based	Australia	20 (1.8)	23 (3.5)	1
BCFR-Philadelphia	clinic-based	North America	60 (5.5)	28 (4.2)	1
BCFR-Utah	clinic-based	North America	36 (3.3)	17 (2.6)	
BCFR-New York	clinic-based	North America	104 (9.5)	32 (4.8)	
BCFR-Ontario	population-based	North America	67 (6.1)	39 (5.9)	
BCFR-Northern California	population-based	North America	31 (2.8)	29 (4.4)	1
National Cancer Institute	clinic-based	North America	81 (7.4)	27 (4.1)	
INHERIT BRCAs-Quebec	multiple-case family-based	Quebec	50 (4.6)	52 (7.9)	
Total			1,090	661	3
Affected with breast cancer †			598 (54.9)	392 (59.3)	3
Affected with ovarian cancer †			83 (7.6)	26 (3.9)	0
Number of families			685	390	3

*Source abbreviations: EMBRACE, Epidemiological Study of Familial Breast Cancer; kConFaB, Kathleen Cunningham Consortium for Research into Familial Breast Cancer; BCFR, Breast Cancer Family Registry, AJBCS, Australian Jewish Breast Cancer study; ABCFS, Australian Breast Cancer Family Study; National Cancer Institute, Cancer Family Registry, Intramural program of the National Cancer Institute; INHERIT BRCAs, Interdisciplinary Health Research International Team on Breast Cancer susceptibility.

† Cancer type refers to first primary cancer diagnosis. One *BRCA2* carrier with breast cancer was censored as unaffected at age of prior mastectomy.

Table 2. Breast cancer risk associated with *AIB1* Gln repeat length

		BRCA1 mutation carriers				*BRCA2* mutation carriers			
		Adjusted for group, ethnicity, and year of birth		Adjusted for group, ethnicity, year of birth, and additional variables		Adjusted for group, ethnicity, and year of birth		Adjusted for group, ethnicity, year of birth, and additional variables	
		P	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)
AIB1 repeat as a categorical variable									
AIB1 ≥28 Gln	unweighted analysis	0.2	0.89 (0.75-1.06)	0.1	0.87 (0.73-1.04)	0.6	1.06 (0.85-1.33)	0.2	1.16 (0.91-1.48)
	weighted analysis	0.1	0.83 (0.65-1.06)	0.03	0.76 (0.59-0.97)	0.3	1.18 (0.84-1.67)	0.1	1.34 (0.92-1.93)
AIB1 ≥29 Gln	unweighted analysis	0.7	0.96 (0.78-1.18)	0.7	0.96 (0.78-1.17)	0.9	1.02 (0.79-1.32)	0.3	1.12 (0.88-1.43)
	weighted analysis	0.6	0.93 (0.71-1.21)	0.7	0.95 (0.73-1.24)	0.8	0.96 (0.65-1.41)	0.4	1.18 (0.82-1.70)
AIB1 repeat as a continuous variable									
AIB1 small allele size	unweighted analysis	0.2	0.95 (0.89-1.02)	0.07	0.94 (0.87-1.01)	0.6	1.02 (0.95-1.10)	0.2	1.06 (0.97-1.15)
	weighted analysis	0.2	0.94 (0.86-1.03)	0.02	0.90 (0.82-0.99)	0.5	1.04 (0.92-1.17)	0.2	1.10 (0.97-1.26)
AIB1 large allele size	unweighted analysis	0.3	1.05 (0.96-1.15)	0.3	1.07 (0.94-1.20)	0.6	0.96 (0.82-1.12)	0.9	0.99 (0.84-1.17)
	weighted analysis	0.3	1.07 (0.95-1.22)	0.2	1.11 (0.94-1.30)	0.2	0.86 (0.68-1.09)	0.6	0.93 (0.72-1.21)
AIB1 average allele size	unweighted analysis	0.7	0.98 (0.89-1.08)	0.4	0.95 (0.85-1.06)	0.8	1.02 (0.90-1.15)	0.3	1.07 (0.94-1.22)
	weighted analysis	0.7	0.97 (0.85-1.11)	0.2	0.91 (0.79-1.06)	0.9	1.02 (0.84-1.22)	0.3	1.12 (0.91-1.37)

NOTE: First primary breast cancer diagnosis was considered an event (status affected), whereas first primary ovarian cancers were censored as unaffected at age of diagnosis, and individuals without breast or ovarian cancer were censored as unaffected at age at interview. All individuals were censored at age of prior prophylactic mastectomy. Mean age of *BRCA1* carriers was 41 years (20-81) for affected individuals, and 42 years (17-83) for individuals censored as unaffected. Mean age of *BRCA2* carriers was 43 years (23-72) for affected individuals, and 45 years (18-84) for individuals censored as unaffected. Individuals carrying both *BRCA1* and *BRCA2* mutations were included in *BRCA1* and *BRCA2* analyses. Analyses were adjusted for source group, ethnicity, year of birth, and hormonal variables oophorectomy, parity, age at menarche, and contraceptive pill use. Categorization for group was as shown in Table 1, and for other variables as described in Spurdle et al. (11). Oophorectomy and parity were treated as time-dependent variables from age at first variable event. Questionnaire information on potential confounders for analyses adjusting for additional hormonal variables was available for 964 *BRCA1* and 598 *BRCA2* carriers.

adjusted also for reproductive factors. Risk estimates using the weighted Cox regression approach were similar to the unweighted estimates as expected when the null hypothesis is true (18).

Risk estimates did not differ materially when women with a first primary diagnosis of ovarian cancer were excluded [e.g., RR (95% CI) for the ≥29 CAG cutpoint of 0.96 (0.79-1.16) for *BRCA1* mutation carriers ($P = 0.7$), and 1.05 (0.83-1.33) for *BRCA2* mutation carriers ($P = 0.7$)], or when carriers ascertained from population-based sites were excluded (data not shown), suggesting that the preferential ascertainment of cases versus controls from these sites did not bias results.

Our sample size was large enough to detect effects reported by Rebbeck et al. (8). Assuming the age distribution of affected and unaffected carriers as shown in Table 1, simulations estimated the power of detecting risk ratios of 1.56 and 2.85 to be 91% and 100%, respectively, for *BRCA1* mutation carriers, and 58% and 100% for *BRCA2* carriers. The upper 95% confidence limits on the RR in our analysis (1.21 for *BRCA1*, 1.70 for *BRCA2* ≥29 repeats based on the weighted analysis) exclude any substantial risk.

Conclusion

Our study found no evidence to support the previously reported associations of *AIB1* glutamine repeat length with increased breast cancer risk in two relatively small studies of *BRCA1* carriers (8, 9), supporting recently published negative findings from a much larger study of mutation carriers (10). Given the large sample size, with more than adequate power to detect previously reported effects, we conclude that the *AIB1* glutamine repeat does not substantially modify risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers.

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